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36

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(71) Applicant: NEUROSCIENCES RESEARCH FOUNDATION, INC. [US/US]; 10640 John Jay Hopkins Drive, San Diego, CA 92121 (US).

(72) Inventors: PIOMELLI, Daniele; 4992 Academy Street, San Diego, CA 92109 (US). BELTRAMO, Massimiliano; Apartment 2609, 7425 Charmant Drive, San Diego, CA 92112 (US).

(74) Agent: DUNCAN, Margaret, M.; McDermott, Will & Emery, Suite 4400, 227 W. Monroe Street, Chicago, IL 60606-5096 (US).

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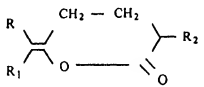
(54) Title: METHODS OF TREATING MENTAL DISEASES, INFLAMMATION AND PAIN



(1)

(57) Abstract

Methods are disclosed for treating or preventing disorders such as mental diseases, inflammation and pain by inhibiting the enzyme anandamide amidohydrolase. A therapeutically effective level of an anandamide amidohydrolase inhibitor is administered such as a therapeutically effective level of a haloenol lactone. Preferably, the haloenol lactone is of formula (I) wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals, derivatives of said haloenol lactones, and mixtures thereof. The haloenol lactone, E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one, is most preferred.

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METHODS OF TREATING MENTAL DISEASES, INFLAMMATION AND PAIN

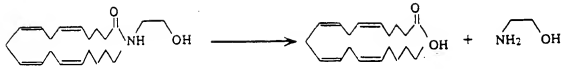
FIELD OF THE INVENTION

The invention relates to methods and compositions for treating disorders such as mental diseases, inflammation and pain. More particularly, the invention relates to methods for treating such disorders by administering a therapeutically effective level of an anandamide amidohydrolase inhibitor.

BACKGROUND OF THE INVENTION

Anandamide (N-arachidonoylethanolamine) is thought to act as an endogenous cannabinoid neurotransmitter in vertebrate nervous systems. It binds to and activates cannabinoid receptors and simulates many distinctive effects typical of plant-derived or synthetic cannabinoid drugs.

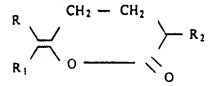
Biochemical evidence indicates that anandamide is produced in and released from neurons in an activity-dependent manner. Further, as expected of a signalling molecule, anandamide is short-lived: its life-span is limited by uptake into neural cells and by enzymatic hydrolysis. Anandamide hydrolysis is catalyzed by the enzyme anandamide amidohydrolase, which converts anandamide to yield two inactive metabolites, arachidonate and ethanolamine. This reaction is illustrated by the following:



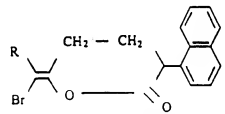
Anandamide amidohydrolase is likely to play an important role in the physiological degradation of anandamide. Three lines of evidence support this possibility. First, anandamide amidohydrolase is highly selective. Second, anandamide amidohydrolase is discretely distributed in the central nervous system, where its localization parallels that of cannabinoid receptors. Third, a protease inhibitor that blocks anandamide amidohydrolase non-selectively, phenylmethylsulphonylfluoride, extends the actions of anandamide.

Therefore, inhibition of anandamide amidohydrolase to increase the accumulation of anandamide at its sites of action is desirable as a potential therapeutic approach for the treatment or prevention of disorders such as mental diseases, inflammation and pain, including treatment or prevention of schizophrenia, mood disorders, anorexia, multiple sclerosis, spasticity and glaucoma. Despite these potential applications, no potent and selective inhibitors of anandamide amidohydrolase have been identified as yet.

The anandamide amidohydrolase inhibitors useful in the present invention comprise haloenol lactones. The preferred haloenol lactones are compounds of the formula:



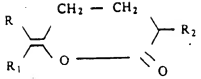
wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals. A most preferred haloenol lactone is E-6-(bromomethylene) tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one which has the following formula:



The synthesis of this compound and the identification of its ability to inhibit an enzyme which is unrelated to anandamide amidohydrolase, i.e., the cardiac calcium-independent phospholipase A_2, have been described in the following patents and publications: Hazen, et al., J. Biol. Chem. 266, 7227-7232 (1991); Weiss, et al., U.S. Patent No. 5,208,244; and Balsinde, et al., Proc. Natl. Acad. Sci. U.S.A. 92, 8527-8531 (1995).

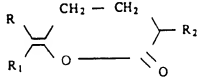
SUMMARY OF THE INVENTION

The invention comprises methods of treating or preventing disorders such as mental diseases, inflammation and pain, including schizophrenia, mood disorders, anorexia, multiple sclerosis, spasticity and glaucoma by administering a therapeutically effective level of an anandamide amidohydrolase inhibitor. The preferred anandamide amidohydrolase inhibitors comprise haloenol lactones. The preferred haloenol lactones are compounds of the formula:



wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals, and derivatives and mixtures thereof. The most preferred anandamide amidohydrolase inhibitors comprise E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one, derivatives of this compound, and mixtures thereof.

The present invention further comprises methods of inhibiting anandamide amidohydrolase by administering a therapeutically effective amount of a haloenol lactone. The preferred haloenol lactones are compounds of the formula:



wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals, derivatives of these compounds and mixtures thereof. The most preferred anandamide amidohydrolase inhibitors comprise E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one.

The invention further comprises pharmaceutical compositions comprising anandamide amidohydrolase inhibitors for treating mental diseases, inflammation and pain, such as schizophrenia, mood disorders, anorexia, multiple sclerosis, spasticity and glaucoma. The preferred compositions comprise a

haloenol lactone at a therapeutically effective level to inhibit anandamide amidohydrolase.

DESCRIPTION OF THE DRAWINGS

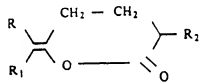
FIGURE 1 is a graph showing a comparison of the effects of a haloenol lactone of the invention on anandamide amidohydrolase activities from rat brain and rat liver;

FIGURES 2A and 2B are graphs showing measurements of the levels of radiolabeled arachidonic acid accumulated in the presence of various concentrations of a haloenol lactone of the invention (Fig. 2A), or levels of phospholipids containing radiolabeled arachidonic acid (Fig. 2B); and

FIGURE 3 is a graph showing that intracellular levels of radiolabeled anandamide were greatly increased in the presence of a haloenol lactone of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The preferred anandamide amidohydrolase inhibitors of the invention are haloenol lactones. The preferred haloenol lactones are compounds of the general formula:



wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals, and derivatives and mixtures thereof. The preferred haloenol

lactones useful in the methods and compositions of the invention include E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one, derivatives of this compound, and mixtures thereof.

Inhibition of anandamide amidohydrolase causes the accumulation of endogenously produced anandamide. Endogenous anandamide, in turn, activates cannabinoid receptors, resulting in therapeutically favorable effects that include mood elevation, appetite stimulation, relief of pain and inflammation, and symptomatic relief in diseases such as multiple sclerosis and glaucoma.

The following examples illustrate the anandamide amidohydrolase inhibitors of the invention.

Example 1

Anandamide amidohydrolase assay

An assay was developed which demonstrated inhibition of rat brain anandamide amidohydrolase by E-6-(bromomethylene) tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one. This assay consisted of determining the amount of radiolabeled arachidonic acid liberated from radiolabeled anandamide by rat brain anandamide amidohydrolase in the presence of various concentrations of E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one. This assay was also used to show that E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one is more effective on brain tissue anandamide amidohydrolase

activity, by examining its effect on rat liver anandamide amidohydrolase.

Anandamide amidohydrolase was measured in rat brain or rat liver microsome fractions. The fractions (0.1 mg of protein) were prepared following the protocols of Desarnaud et al., J. Biol. Chem. 270, 6030-6035 (1995), and were incubated in 50 mM Tris-Cl (pH 7.4) at 37°C, in the presence of radiolabeled anandamide obtained from New England Nuclear, Wilmington, DE, 221 Ci/mmol), plus various concentrations of test inhibitor (0.1-100 μM). After 10 min. of incubation, the reactions were stopped with cold methanol, the radiolabeled lipids extracted with chloroform, and the organic phases brought to dryness under a stream of N_2 gas. The radioactive products were then fractionated by thin-layer chromatography (solvent system: chloroform/methanol/ammonia, 90:10:1 vol/vol/vol), collected by scraping appropriate areas of the chromatography plate, and quantified by liquid scintillation counting.

The effects of E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one on anandamide amidohydrolases from rat brain or liver are shown in Figure 1. This compound is potent in inhibiting brain anandamide amidohydrolase. The concentration of E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one which decreases the enzyme activity to 50% of the activity measured in the absence of the compound (defined as IC_{50}), was 0.7 μM.

Underscoring the tissue differences of this inhibitory effect, inhibition of the liver enzyme was achieved at concentrations of E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one that were more than 100-fold higher than in brain (IC_{50}=97 μM).

Pharmaceutical compositions comprising the haloenol lactones of the invention can be administered utilizing an effective inhibitory amount of the compound(s). This amount can range from about 1 nM to 0.1 mM, preferably from about 1 μM to about 50 μM. A most preferred effective amount is about 10 μM. Such compositions can be prepared with acceptable diluents and/or carriers, as described, for example, in Remington's Pharmaceutical Sciences, Arthur Osol, Ed., 16th Ed., 1980, Mack Publishing Company.

Example 2

Assay in cultures of cortical astrocytes

An additional assay demonstrated inhibition of anandamide amidohydrolase in intact neural cells. This assay consisted of determining the amount of radiolabeled arachidonic acid produced, when cultures of rat cortical astrocytes were incubated in the presence of radiolabeled anandamide.

Cultures of rat cortical astrocytes, essentially free of neurons, were prepared following the standard procedures described in Cadas et al., J. Neurosci. 16, 3934-3942 (1996), and used after 3 weeks in culture. The cultures were incubated in

Krebs Tris solution (pH 7.4) at 37°C, in the presence of radiolabeled anandamide plus various concentrations of E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one (0.1-100 μM). After 20 min. of incubation, the reactions were stopped with cold methanol, and the cells were scraped from the culture dishes and subjected to chloroform extraction. The organic phases were dried, and analyzed as follows. To measure radiolabeled anandamide and arachidonic acid, the organic extracts were fractionated by silica gel G column chromatography, as described in Fontana et al., Prostaglandins Leukotrienes Essential Fatty Acids 53, 301-308 (1995). Radiolabeled anandamide and arachidonic acid were eluted from the column with a solvent system of chloroform/methanol (9:1, vol/vol), and further purified by thin-layer chromatography (solvent system of chloroform/methanol/ammonia, 80:20:1, vol/vol/vol). To measure radiolabeled phospholipids, which were formed in intact cells from the enzymatic esterification of radiolabeled arachidonic acid, the organic extracts were fractionated by thin-layer chromatography (solvent system of chloroform/methanol/ammonia/water, 65:25:4:1, vol/vol/vol/vol).

E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one is potent in inhibiting the anandamide amidohydrolase of intact astrocytes (IC_{50} = 0.5 uM). This can be shown either by measuring the levels of radiolabeled arachidonic acid accumulated in the presence of various concentrations of the inhibitor (Fig. 2A), or by measuring the levels of phospholipids

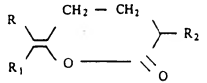
containing radiolabeled arachidonic acid (Fig. 2B). By contrast, E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one does not inhibit the uptake of radiolabeled anandamide. This is indicated by the fact that the intracellular levels of radiolabeled anandamide were greatly increased in the presence of this compound, which would not be expected if the uptake were inhibited (Fig. 3).

The embodiments of the invention disclosed herein have been discussed for the purpose of familiarizing the reader with novel aspects of the invention. Although preferred embodiments of the invention have been shown and described, many changes, modifications, and substitutions may be made by one having skill in the art without necessarily departing from the spirit and scope of the invention.

CLAIMS

1. A method of inhibiting anandamide amidohydrolase by administering a therapeutically effective amount of a haloenol lactone.

2. The method of claim 1 wherein the haloenol lactone comprises a compound of the formula:



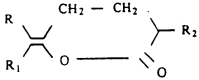
wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals, derivatives and mixtures thereof.

3. The method of claim 1 wherein said haloenol lactone comprises E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one.

4. A method of treating mental disease, inflammation or pain comprising administering a therapeutically effective level of an anandamide amidohydrolase inhibitor.

5. The method of claim 4 wherein the anandamide amidohydrolase inhibitor comprises a haloenol lactone.

6. The method of claim 4 wherein the haloenol lactone comprises a compound of the formula:

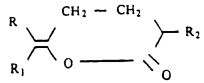


wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals, derivatives of said haloenol lactones, and mixtures thereof.

7. The method of claim 4 wherein the haloenol lactone comprises E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one.

8. A composition for treating mental disease, inflammation or pain comprising a therapeutically effective level of a haloenol lactone sufficient to inhibit anandamide amidohydrolase and a pharmaceutically acceptable carrier.

9. The composition of claim 8 wherein the haloenol lactone comprises a compound of the formula:



wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals, derivatives of said haloenol lactones, and mixtures thereof.

10. The composition of claim 8 wherein the haloenol lactone comprises E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one.

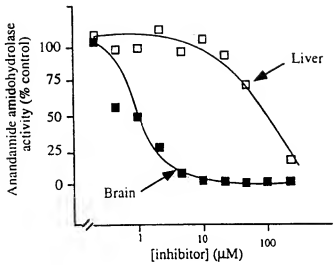


Figure 1

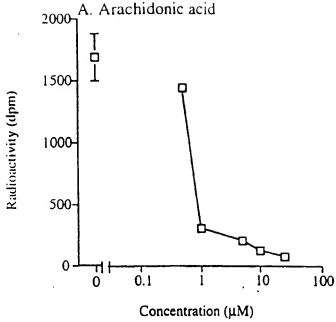


Figure 2A

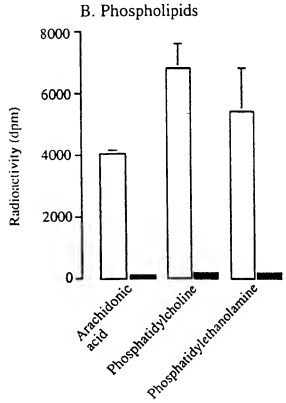


Figure 2B

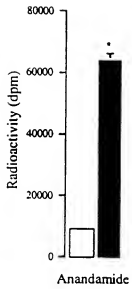


Figure 3

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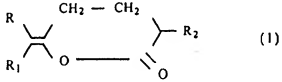

(1)

(57) Abstract

Methods are disclosed for treating or preventing disorders such as mental diseases, inflammation and pain by inhibiting the enzyme anandamide amidohydrolase. A therapeutically effective level of an anandamide amidohydrolase inhibitor is administered such as a therapeutically effective level of a haloenol lactone. Preferably, the haloenol lactone is of formula (I) wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals, derivatives of said haloenol lactones, and mixtures thereof. The haloenol lactone, E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one, is most preferred.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/22063

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) : A61K 31/35
US CL : 514/460
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/460

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE on STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	US 4,602,006 A (KRANTZ ET AL.) 22 July 1986, col. 16, lines 55-70.	1-3, 8-10 --- 4-7
X --- A	US 5,208,244 A (WEISS ET AL.) 04 May 1993, col. 2, lines 33-50 and claims 1-15.	4-10 --- 1-3
X --- A	Database CAPLUS on STN, AN 1996:403231, Mallet et al, 'The endogenous cannabinoid receptor agonist anandamide impairs memory in rats,' abstract, Behav. Pharmacol. (1996), 7(3), 276-284.	4 --- 1-3 and 5-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search 14 JANUARY 1998	Date of mailing of the international search report 0 6 JUL 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KEITH MACMILLAN Telephone No. (703) 308-1235

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(71) Applicant: NEUROSCIENCES RESEARCH FOUNDATION, INC. [US/US]; 10640 John Jay Hopkins Drive, San Diego, CA 92121 (US).

(72) Inventors: PIOMELLI, Daniele; 4992 Academy Street, San Diego, CA 92109 (US). BELTRAMO, Massimiliano; Apartment 2609, 7425 Charmant Drive, San Diego, CA 92112 (US).

(74) Agent: DUNCAN, Margaret, M.; McDermott, Will & Emery, Suite 4400, 227 W. Monroe Street, Chicago, IL 60606-5096 (US).

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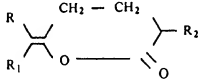
(54) Title: METHODS OF TREATING MENTAL DISEASES, INFLAMMATION AND PAIN



(1)

(57) Abstract

Methods are disclosed for treating or preventing disorders such as mental diseases, inflammation and pain by inhibiting the enzyme anandamide amidohydrolase. A therapeutically effective level of an anandamide amidohydrolase inhibitor is administered such as a therapeutically effective level of a haloenol lactone. Preferably, the haloenol lactone is of formula (I) wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals, derivatives of said haloenol lactones, and mixtures thereof. The haloenol lactone, E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyran-2-one, is most preferred.

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METHODS OF TREATING MENTAL DISEASES, INFLAMMATION AND PAIN

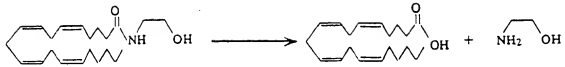
FIELD OF THE INVENTION

The invention relates to methods and compositions for treating disorders such as mental diseases, inflammation and pain. More particularly, the invention relates to methods for treating such disorders by administering a therapeutically effective level of an anandamide amidohydrolase inhibitor.

BACKGROUND OF THE INVENTION

Anandamide (N-arachidonoylethanolamine) is thought to act as an endogenous cannabinoid neurotransmitter in vertebrate nervous systems. It binds to and activates cannabinoid receptors and simulates many distinctive effects typical of plant-derived or synthetic cannabinoid drugs.

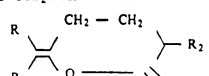
Biochemical evidence indicates that anandamide is produced in and released from neurons in an activity-dependent manner. Further, as expected of a signalling molecule, anandamide is short-lived: its life-span is limited by uptake into neural cells and by enzymatic hydrolysis. Anandamide hydrolysis is catalyzed by the enzyme anandamide amidohydrolase, which converts anandamide to yield two inactive metabolites, arachidonate and ethanolamine. This reaction is illustrated by the following:



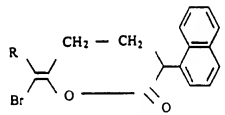
Anandamide amidohydrolase is likely to play an important role in the physiological degradation of anandamide. Three lines of evidence support this possibility. First, anandamide amidohydrolase is highly selective. Second, anandamide amidohydrolase is discretely distributed in the central nervous system, where its localization parallels that of cannabinoid receptors. Third, a protease inhibitor that blocks anandamide amidohydrolase non-selectively, phenylmethylsulphonylfluoride, extends the actions of anandamide.

Therefore, inhibition of anandamide amidohydrolase to increase the accumulation of anandamide at its sites of action is desirable as a potential therapeutic approach for the treatment or prevention of disorders such as mental diseases, inflammation and pain, including treatment or prevention of schizophrenia, mood disorders, anorexia, multiple sclerosis, spasticity and glaucoma. Despite these potential applications, no potent and selective inhibitors of anandamide amidohydrolase have been identified as yet.

The anandamide amidohydrolase inhibitors useful in the present invention comprise haloenol lactones. The preferred haloenol lactones are compounds of the formula:



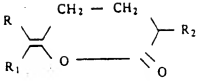
wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals. A most preferred haloenol lactone is E-6-(bromomethylene) tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one which has the following formula:



The synthesis of this compound and the identification of its ability to inhibit an enzyme which is unrelated to anandamide amidohydrolase, i.e., the cardiac calcium-independent phospholipase A_2, have been described in the following patents and publications: Hazen, et al., J. Biol. Chem. 266, 7227-7232 (1991); Weiss, et al., U.S. Patent No. 5,208,244; and Balsinde, et al., Proc. Natl. Acad. Sci. U.S.A. 92, 8527-8531 (1995).

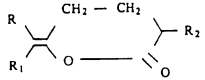
SUMMARY OF THE INVENTION

The invention comprises methods of treating or preventing disorders such as mental diseases, inflammation and pain, including schizophrenia, mood disorders, anorexia, multiple sclerosis, spasticity and glaucoma by administering a therapeutically effective level of an anandamide amidohydrolase inhibitor. The preferred anandamide amidohydrolase inhibitors comprise haloenol lactones. The preferred haloenol lactones are compounds of the formula:



wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals, and derivatives and mixtures thereof. The most preferred anandamide amidohydrolase inhibitors comprise E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one, derivatives of this compound, and mixtures thereof.

The present invention further comprises methods of inhibiting anandamide amidohydrolase by administering a therapeutically effective amount of a haloenol lactone. The preferred haloenol lactones are compounds of the formula:



wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals, derivatives of these compounds and mixtures thereof. The most preferred anandamide amidohydrolase inhibitors comprise E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one.

The invention further comprises pharmaceutical compositions comprising anandamide amidohydrolase inhibitors for treating mental diseases, inflammation and pain, such as schizophrenia, mood disorders, anorexia, multiple sclerosis, spasticity and glaucoma. The preferred compositions comprise a

haloenol lactone at a therapeutically effective level to inhibit anandamide amidohydrolase.

DESCRIPTION OF THE DRAWINGS

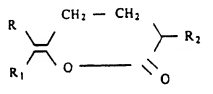
FIGURE 1 is a graph showing a comparison of the effects of a haloenol lactone of the invention on anandamide amidohydrolase activities from rat brain and rat liver;

FIGURES 2A and 2B are graphs showing measurements of the levels of radiolabeled arachidonic acid accumulated in the presence of various concentrations of a haloenol lactone of the invention (Fig. 2A), or levels of phospholipids containing radiolabeled arachidonic acid (Fig. 2B); and

FIGURE 3 is a graph showing that intracellular levels of radiolabeled anandamide were greatly increased in the presence of a haloenol lactone of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The preferred anandamide amidohydrolase inhibitors of the invention are haloenol lactones. The preferred haloenol lactones are compounds of the general formula:



wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals, and derivatives and mixtures thereof. The preferred haloenol

lactones useful in the methods and compositions of the invention include E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one, derivatives of this compound, and mixtures thereof.

Inhibition of anandamide amidohydrolase causes the accumulation of endogenously produced anandamide. Endogenous anandamide, in turn, activates cannabinoid receptors, resulting in therapeutically favorable effects that include mood elevation, appetite stimulation, relief of pain and inflammation, and symptomatic relief in diseases such as multiple sclerosis and glaucoma.

The following examples illustrate the anandamide amidohydrolase inhibitors of the invention.

Example 1

Anandamide amidohydrolase assay

An assay was developed which demonstrated inhibition of rat brain anandamide amidohydrolase by E-6-(bromomethylene) tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one. This assay consisted of determining the amount of radiolabeled arachidonic acid liberated from radiolabeled anandamide by rat brain anandamide amidohydrolase in the presence of various concentrations of E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one. This assay was also used to show that E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one is more effective on brain tissue anandamide amidohydrolase

activity, by examining its effect on rat liver anandamide amidohydrolase.

Anandamide amidohydrolase was measured in rat brain or rat liver microsome fractions. The fractions (0.1 mg of protein) were prepared following the protocols of Desarnaud et al., J. Biol. Chem. 270, 6030-6035 (1995), and were incubated in 50 mM Tris-Cl (pH 7.4) at 37°C, in the presence of radiolabeled anandamide obtained from New England Nuclear, Wilmington, DE, 221 Ci/mmol), plus various concentrations of test inhibitor (0.1-100 μM). After 10 min. of incubation, the reactions were stopped with cold methanol, the radiolabeled lipids extracted with chloroform, and the organic phases brought to dryness under a stream of N_2 gas. The radioactive products were then fractionated by thin-layer chromatography (solvent system: chloroform/methanol/ammonia, 90:10:1 vol/vol/vol), collected by scraping appropriate areas of the chromatography plate, and quantified by liquid scintillation counting.

The effects of E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one on anandamide amidohydrolases from rat brain or liver are shown in Figure 1. This compound is potent in inhibiting brain anandamide amidohydrolase. The concentration of E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one which decreases the enzyme activity to 50% of the activity measured in the absence of the compound (defined as IC_{50}), was 0.7 μM.

Underscoring the tissue differences of this inhibitory effect, inhibition of the liver enzyme was achieved at concentrations of E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one that were more than 100-fold higher than in brain (IC_{50}=97 μM).

Pharmaceutical compositions comprising the haloenol lactones of the invention can be administered utilizing an effective inhibitory amount of the compound(s). This amount can range from about 1 nM to 0.1 mM, preferably from about 1 μM to about 50 μM. A most preferred effective amount is about 10 μM. Such compositions can be prepared with acceptable diluents and/or carriers, as described, for example, in Remington's Pharmaceutical Sciences, Arthur Osol, Ed., 16th Ed., 1980, Mack Publishing Company.

Example 2

Assay in cultures of cortical astrocytes

An additional assay demonstrated inhibition of anandamide amidohydrolase in intact neural cells. This assay consisted of determining the amount of radiolabeled arachidonic acid produced, when cultures of rat cortical astrocytes were incubated in the presence of radiolabeled anandamide.

Cultures of rat cortical astrocytes, essentially free of neurons, were prepared following the standard procedures described in Cadas et al., J. Neurosci. 16, 3934-3942 (1996), and used after 3 weeks in culture. The cultures were incubated in

Krebs Tris solution (pH 7.4) at 37°C, in the presence of radiolabeled anandamide plus various concentrations of E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one (0.1-100 μM). After 20 min. of incubation, the reactions were stopped with cold methanol, and the cells were scraped from the culture dishes and subjected to chloroform extraction. The organic phases were dried, and analyzed as follows. To measure radiolabeled anandamide and arachidonic acid, the organic extracts were fractionated by silica gel G column chromatography, as described in Fontana et al., Prostaglandins Leukotrienes Essential Fatty Acids 53, 301-308 (1995). Radiolabeled anandamide and arachidonic acid were eluted from the column with a solvent system of chloroform/methanol (9:1, vol/vol), and further purified by thin-layer chromatography (solvent system of chloroform/methanol/ammonia, 80:20:1, vol/vol/vol). To measure radiolabeled phospholipids, which were formed in intact cells from the enzymatic esterification of radiolabeled arachidonic acid, the organic extracts were fractionated by thin-layer chromatography (solvent system of chloroform/methanol/ammonia/water, 65:25:4:1, vol/vol/vol/vol).

E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one is potent in inhibiting the anandamide amidohydrolase of intact astrocytes (IC_{50} = 0.5 uM). This can be shown either by measuring the levels of radiolabeled arachidonic acid accumulated in the presence of various concentrations of the inhibitor (Fig. 2A), or by measuring the levels of phospholipids

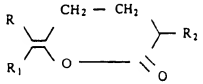
containing radiolabeled arachidonic acid (Fig. 2B). By contrast, E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one does not inhibit the uptake of radiolabeled anandamide. This is indicated by the fact that the intracellular levels of radiolabeled anandamide were greatly increased in the presence of this compound, which would not be expected if the uptake were inhibited (Fig. 3).

The embodiments of the invention disclosed herein have been discussed for the purpose of familiarizing the reader with novel aspects of the invention. Although preferred embodiments of the invention have been shown and described, many changes, modifications, and substitutions may be made by one having skill in the art without necessarily departing from the spirit and scope of the invention.

CLAIMS

1. A method of inhibiting anandamide amidohydrolase by administering a therapeutically effective amount of a haloenol lactone.

2. The method of claim 1 wherein the haloenol lactone comprises a compound of the formula:



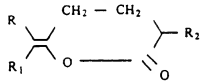
wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals, derivatives and mixtures thereof.

3. The method of claim 1 wherein said haloenol lactone comprises E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one.

4. A method of treating mental disease, inflammation or pain comprising administering a therapeutically effective level of an anandamide amidohydrolase inhibitor.

5. The method of claim 4 wherein the anandamide amidohydrolase inhibitor comprises a haloenol lactone.

6. The method of claim 4 wherein the haloenol lactone comprises a compound of the formula:

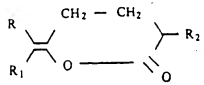


wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals, derivatives of said haloenol lactones, and mixtures thereof.

7. The method of claim 4 wherein the haloenol lactone comprises E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one.

8. A composition for treating mental disease, inflammation or pain comprising a therapeutically effective level of a haloenol lactone sufficient to inhibit anandamide amidohydrolase and a pharmaceutically acceptable carrier.

9. The composition of claim 8 wherein the haloenol lactone comprises a compound of the formula:



wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals, derivatives of said haloenol lactones, and mixtures thereof.

10. The composition of claim 8 wherein the haloenol lactone comprises E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one.

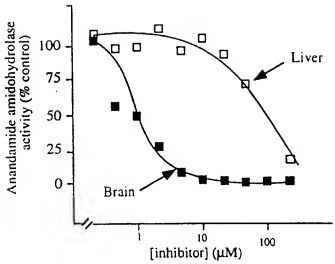


Figure 1

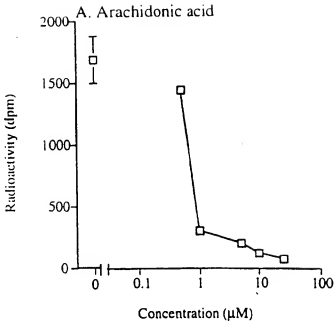


Figure 2A

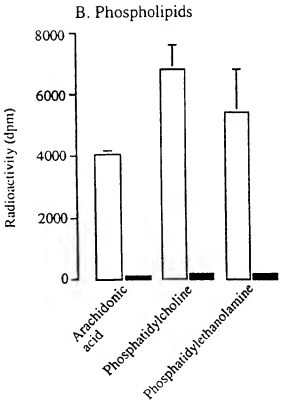


Figure 2B

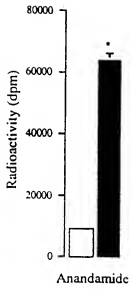


Figure 3

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicant: NEUROSCIENCES RESEARCH FOUNDATION, INC. [US/US]; 10640 John Jay Hopkins Drive, San Diego, CA 92121 (US).

(72) Inventors: PIOMELLI, Daniele; 4992 Academy Street, San Diego, CA 92109 (US). BELTRAMO, Massimiliano; Apartment 2609, 7425 Charmant Drive, San Diego, CA 92112 (US).

(74) Agent: DUNCAN, Margaret, M.; McDermott, Will & Emery, Suite 4400, 227 W. Monroe Street, Chicago, IL 60606-5096 (US).

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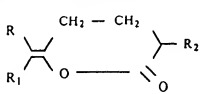

(1)

(57) Abstract

Methods are disclosed for treating or preventing disorders such as mental diseases, inflammation and pain by inhibiting the enzyme anandamide amidohydrolase. A therapeutically effective level of an anandamide amidohydrolase inhibitor is administered such as a therapeutically effective level of a haloenol lactone. Preferably, the haloenol lactone is of formula (I) wherein R is hydrogen, R_1 is a halogen, and R_2 is selected from the group consisting of aryl, aryloxy, and heteroaryl radicals, derivatives of said haloenol lactones, and mixtures thereof. The haloenol lactone, E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyrane-2-one, is most preferred.

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INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/35
US CL : 514/460

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/460

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE on STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	US 4,602,006 A (KRANTZ ET AL.) 22 July 1986, col. 16, lines 55-70.	1-3, 8-10 --- 4-7
X --- A	US 5,208,244 A (WEISS ET AL.) 04 May 1993, col. 2, lines 33-50 and claims 1-15.	4-10 --- 1-3
X --- A	Database CAPLUS on STN, AN 1996:403231, Mallet et al, 'The endogenous cannabinoid receptor agonist anandamide impairs memory in rats,' abstract, Behav. Pharmacol. (1996), 7(3), 276-284.	4 --- 1-3 and 5-10

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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KEITH MACMILLAN Telephone No. (703) 308-1235

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